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Cholesteryl ester transfer in patients with renal failure or renal transplants

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Cholesteryl ester transfer activity in patients with renal failure or renal transplants. Plasma newly-synthesized cholesteryl ester transfer (NCET) rate and concentrations of lipids, lipoproteins and apolipoproteins A1 and B were measured in chronic renal failure patients (dialysis independent and dialysis dependent), patients with a functioning renal transplant and in healthy control subjects with comparable ages and plasma triglycerides. Plasma NCET rates and apoB concentrations were significantly higher in patients treated by continuous ambulatory peritoneal dialysis (CAPD) compared with controls. In normolipidemic subjects (cholesterol < 6.5 mmol/liter, triglycerides < 2.0 mmol/liter), plasma NCET rates did not differ significantly from rates in the corresponding control subjects. In hyperlipidemic subjects, plasma NCET rates were significantly higher than rates in the normolipidemic subgroup. Plasma NCET rates were correlated closely with plasma apoB levels in all renal patients combined ($r = 0.754$, $N = 53$, $P < 0.001$) and with plasma cholesteryl ester mass transfer ($r = 0.853$, $N = 13$, $P < 0.001$). We conclude that, in the absence of hyperlipidemia, plasma NCET rate is normal in patients with chronic renal failure irrespective of the treatment for uremia, and when hyperlipidemia is present NCET rates are raised and may contribute to elevated levels of the proatherogenic apoB-containing lipoproteins.

Initial steps in the transport of cholesterol from peripheral tissues to the liver (reverse cholesterol transport) include binding of cell cholesterol by high density lipoproteins (HDL), esterification of free cholesterol by lecithin:cholesterol acyltransferase (LCAT) activity associated with HDL, and transfer of cholesteryl esters formed in HDL to triglyceride-rich lipoproteins [1, 2]. Cholesteryl ester transfer protein (CETP) catalyzes the redistribution of newly synthesized cholesteryl esters from HDL to other lipoproteins in plasma [3]. A substantial proportion of the cholesteryl esters formed in the LCAT reaction are transferred to triglyceride-rich lipoproteins and low density lipoproteins (LDL) [4]. When hepatic LDL receptors are down-regulated, transfer of cholesteryl esters into apoB-containing lipoproteins is thought to result in accumulation of cholesteryl ester-enriched LDL particles and very low density lipoprotein (VLDL) remnants in the circulation, which can promote atherosclerosis [3]. On the other hand, if hepatic clearance of apoB-containing lipoproteins is efficient then transfer of cholesteryl esters to these lipoproteins, which are largely catabolised by the liver [5], may be an effective pathway for removing excess cell cholesterol from the body. Thus, the quantity of newly synthesized cholesteryl esters transferred into apoB-containing lipoproteins could influence the rate of reverse cholesterol transport. Efficient reverse cholesterol transport is believed to prevent accumulation of cholesterol in arterial tissue and the development of atherosclerosis.

Patients with chronic renal failure [6] and renal transplant recipients [7] have increased risk of coronary heart disease. In patients treated by hemodialysis, there is evidence that reverse cholesterol transport is deranged with abnormal transport of cholesterol between cultured fibroblasts and plasma such that cholesterol moves from plasma to cells [8]. Also, plasma cholesterol esterification and transfer of cholesteryl esters from HDL are greatly reduced [8, 9]. The low rate of cholesteryl ester transfer in hemodialysis plasma is due to the low rate of cholesteryl esters synthesized by LCAT activity and the inefficient transfer of these esters to apoB-containing lipoproteins of abnormal composition [8]. In contrast, these defects are not present in plasma from comparable patients treated by continuous ambulatory peritoneal dialysis (CAPD) [8]. Evidently the type of treatment used to counteract uremia can influence intravascular cholesterol metabolism. There is, however, little information available on intraplasmatic cholesteryl ester transfer in dialysis independent patients with chronic renal failure and renal transplant recipients. Kidney transplantation, with a return to normal renal function, corrects the deficiency in plasma LCAT activity associated with uremia [10, 11]. However, immunosuppressive drugs taken to maintain the grafts raise plasma lipid levels [12–14], and hyperlipidemia accelerates plasma cholesteryl ester transfer [15, 16]. On the other hand, corticosteroids, which are a component of immunosuppressive therapy, normalize elevated levels of plasma CETP in patients with nephrotic syndrome and normal renal function [17]. Thus, the effect of treatments used in renal failure on the transfer of newly synthesized cholesteryl esters from HDL to apoB-containing lipoproteins warrants investigation. The aim of the present study was therefore to document plasma newly synthesized cholesteryl ester transfer (NCET) rates in both dialysis independent and dialysis dependent patients with chronic renal failure and in renal transplant recipients, considering separately those with hyperlipidemia. Plasma NCET is determined by measuring the accumulation in apoB-containing lipoproteins of cholesteryl esters generated from radiolabeled plasma-free cholesterol, and is therefore an indicator of activity in

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the segment of reverse cholesterol transport involving LCAT and CETP.

Methods

Subjects

Fifty-three patients with chronic renal failure or kidney grafts (ages 15 to 77 years) were recruited from patients attending the Otago Nephrology Unit. Patients with diabetic nephropathy, the nephrotic syndrome or those receiving lipid-lowering therapy were excluded. Thirteen patients were dialysis independent, 12 were receiving hemodialysis, 11 were treated by CAPD and 17 patients were renal transplant recipients. The etiology of renal failure in the patients included glomerulonephritis ($N = 32$), reflux nephropathy ($N = 8$), hypertension ($N = 7$), autosomal-dominant polycystic kidney disease ($N = 2$), and other causes ($N = 4$). Eleven patients (1 dialysis independent, 8 CAPD and 2 hemodialysis) had evidence of ischemic heart disease. Ischemic heart disease was diagnosed on the basis of clinical symptoms and signs along with ECG and/or echocardiographic changes. Twenty-two patients were receiving cardiovascular medications. Where used, antihypertensive therapy was most commonly an angiotensin converting enzyme (ACE) inhibitor and/or dihydropyridine calcium channel antagonist. Only one patient was taking a beta-blocking drug. The majority of dialysis patients were taking calcium carbonate as a phosphate binder and 1 alpha-calcitriol supplementation. Two dialysis patients were receiving erythropoietin subcutaneously. In the renal transplant recipients immunosuppression was with either azathioprine and prednisone ($N = 5$) or triple therapy with cyclosporin A, azathioprine and prednisone ($N = 12$). One patient on hemodialysis was also receiving azathioprine and prednisone for an underlying vasculitis. Patients receiving dialysis were either on home hemodialysis or CAPD. Hemodialysis patients were dialysing three times weekly (12–18 hr/week) using cellulose acetate hollow fiber dialyzers and acetate dialysate. Patients on CAPD used the Baxter Disconnect system. Dietary protein intake was not restricted in any patient group. Apart from advice to hemodialysis patients to restrict intake of potassium-rich foods and fluid intake where necessary, dialysis patients were not given any specific dietary advice. Six patients were smokers (three on hemodialysis and three renal transplant recipients).

Twenty-seven healthy subjects with ages and plasma lipid levels comparable with those of the renal patients were recruited from the population of Dunedin. None was taking any medication and three smoked.

The study was approved by the Otago Area Health Board Ethics Committee and participants gave written and informed consent.

Methods

Blood was taken from subjects after an overnight fast and immediately before the dialysis session in the case of patients treated by hemodialysis. The blood was collected in tubes containing disodium EDTA (1.5 mg/ml) and plasma was separated by low-speed centrifugation at 4°C. VLDL were separated by ultracentrifuging plasma according to the Lipid Research Clinic's protocol [18]. High density lipoprotein cholesterol (HDL-C) was measured in the supernatant after precipitation of apoB-containing lipoproteins with dextran sulphate/magnesium chloride [19]. HDL_3-C was measured in the supernatant after treating plasma with polyethyleneglycol and pelleting the precipitate by centrifugation [20]. Concentrations of low density lipoproteins cholesterol (LDL-C) and HDL_2-C were calculated by difference. The LDL-C contains cholesterol from intermediate density lipoproteins (IDL). Cholesterol, free cholesterol and triglycerides in plasma and plasma fractions were measured using enzymatic kits and calibrators from Boehringer Mannheim (Germany). Concentrations of apoA1 and apoB were determined by immunoturbidimetry [21] using materials from Boehringer Mannheim. Plasma NCET was determined in duplicate by a radioisotope method using endogenous plasma lipoproteins [22]. Briefly, an aliquot of a [^{3}H] cholesterol-albumin emulsion was added to plasma and the mixture was incubated at 4°C to equilibrate the radiolabelled cholesterol with plasma free cholesterol. The mixture was then incubated at 37°C for three hours. At the end of the incubation period VLDL and LDL were precipitated by the addition of phosphotungstate/magnesium chloride. The precipitate was extracted for lipids, the cholesteryl ester and free cholesterol fractions were separated by thin layer chromatography and radioactivity in the fractions was measured. Determination of plasma free cholesterol concentration and total radioactivity added allowed the expression of NCET in nmol/ml/hr. There is no significant esterification of free cholesterol in the VLDL and LDL fractions during the assay [22]. Aliquots of plasma for measurement of NCET were stored briefly (approximately one month at −80°C). Plasma CETP activity is reported as stable for several months at −20°C [23]. Plasma cholesteryl ester mass transfer rates were measured essentially as described previously [24] excepting iodoacetate (1 mmol/liter) was used to inhibit plasma LCAT activity [25]. The rate of cholesterol esterification in whole blood was estimated by incubating aliquots of whole blood at 37°C and 4°C for 10 hours, determining plasma cholesteryl ester concentration (as the difference between total and free cholesterol measurements) at the end of the incubation period and calculating the increase in plasma cholesteryl ester content by subtracting measurements at 4°C from those at 37°C. The presence of blood cells does not influence plasma cholesterol esterification rates [26]. Plasma creatinine concentration was measured by routine automated methods in the laboratories of Dunedin Public Hospital.

Statistical analysis

Comparison of values among groups were made using analysis of variances combined with Tukey's test. Pearson's product moment correlation coefficients were used to test for relationships between variables and partial correlation analysis was used to control for the influence of a third variable. Two-tailed tests of significance were used and a P value of less than or equal to 0.05 was considered to be statistically significant.

Results

Characteristics of the patients with renal disease and the control subjects are shown in Table 1. Apart from renal transplant recipients who were younger, the ages were comparable for patients in each treatment modality and with control subjects.

Rates of NCET (measured in triplicate) and cholesteryl ester mass transfer (measured three times with quadruplicate cholesterol and free cholesterol estimations) were compared in fresh plasma from 11 healthy subjects and two patients regularly receiving hemodialysis (cholesterol: 6.57 mmol/liter and 4.87

Table 1. Clinical characteristics of patients with renal disease and control subjects

	Chronic renal failure					
	DI $N = 13$	HD $N = 12$	CAPD $N = 11$	RT $N = 17$	Controls $N = 27$	P
Age *years*	56 ± 14	45 ± 13	65 ± 7	38 ± 14[a]	53 ± 9	<0.001
Gender (M:F)	8:5	7:5	9:2	9:8	13:14	
Body weight *kg*	65.4 ± 13.5	74.0 ± 13.0	74.4 ± 8.1	68.9 ± 13.2	74.9 ± 13.0	NS
BMI *kg/m*	23.7 ± 3.3	24.6 ± 3.7	26.4 ± 3.1	24.0 ± 3.1	25.4 ± 2.5	NS
Creatinine *μmol/liter*	429 ± 279[ace]	769 ± 352[a]	680 ± 231[a]	139 ± 79[bdf]	90 ± 11	<0.001
Duration of treatment *months*	60 ± 51	48 ± 32	13 ± 10	58 ± 65		0.054

Values are mean ± SD.

Abbreviations are: DI, dialysis independent, HD hemodialysis: CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplant recipients; NS, not significant.

Significant differences by one-way analysis of variances: [a]versus controls; [b]HD versus RT; [c]DI versus HD; [d]RT versus CAPD; [e]DI versus CAPD; [f]RT versus DI.

Table 2. Plasma newly synthesized cholesteryl ester transfer (NCET) rate and lipoprotein profile in patients with renal disease and control subjects

	Chronic renal failure					
	DI $N = 13$	HD $N = 12$	CAPD $N = 11$	RT $N = 17$	Controls $N = 27$	P
Plasma						
NCET rate *nmol/ml/hr*	25.5 ± 10.5	24.4 ± 10.5	32.4 ± 12.5[a]	27.9 ± 10.4	22.1 ± 7.9	0.05
TC *mmol/liter*	6.06 ± 1.68	6.17 ± 1.56	7.43 ± 1.46	6.62 ± 1.36	6.34 ± 0.78	NS
FC *mmol/liter*	1.42 ± 0.43	1.53 ± 0.47	1.58 ± 0.53	1.58 ± 0.44	1.54 ± 0.21	NS
TG *mmol/liter*	1.69 ± 0.63	2.07 ± 1.13	2.19 ± 0.94	1.72 ± 0.81	1.77 ± 0.77	NS
Apo A1 *g/liter*	1.26 ± 0.29	1.34 ± 0.17	1.25 ± 0.14	1.38 ± 0.3	1.56 ± 0.59	NS
Apo B *g/liter*	0.97 ± 0.31	0.95 ± 0.32	1.33 ± 0.34[abcd]	0.99 ± 0.30	0.87 ± 0.20	<0.001
Lipoprotein lipids *mmol/liter*						
VLDL-TC	0.68 ± 0.39	0.86 ± 0.71	0.93 ± 0.65	0.61 ± 0.51	0.61 ± 0.51	NS
VLDL-CE	0.42 ± 0.23	0.48 ± 0.37	0.57 ± 0.40	0.34 ± 0.28	0.38 ± 0.32	NS
VLDL-FC	0.26 ± 0.16	0.39 ± 0.34	0.41 ± 0.26	0.28 ± 0.23	0.23 ± 0.18	NS
VLDL-TG	1.01 ± 0.51	1.40 ± 1.01	1.27 ± 0.74	1.09 ± 0.70	0.91 ± 0.59	NS
LDL-C	4.09 ± 1.45	3.95 ± 1.04	5.15 ± 1.01	4.42 ± 1.15	4.32 ± 0.74	0.072
HDL-C	1.26 ± 0.42	1.29 ± 0.36	1.22 ± 0.24	1.53 ± 0.48	1.53 ± 0.39	0.056
HDL_2-C	0.54 ± 0.30	0.53 ± 0.24	0.51 ± 0.23	0.63 ± 0.36	0.73 ± 0.31	NS
HDL_3-C	0.72 ± 0.22	0.75 ± 0.18	0.71 ± 0.13	0.90 ± 0.18[ce]	0.80 ± 0.12	0.015

Values are mean ± SD.

Abbreviations are: DI dialysis independent; HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplant recipients; NS, not significant; NCET, newly synthesized cholesteryl ester transfer; TC, total cholesterol; FC, free cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Significant difference by one-way analysis of variances are: [a]versus controls; [b]CAPD versus HD; [c]CAPD versus RT; [d]CAPD versus DI; [e]RT versus DI.

mmol/liter; triglycerides: 1.46 mmol/liter and 2.27 mmol/liter, respectively). Plasma NCET was correlated significantly (r = 0.853, N = 13, $P < 0.001$) with plasma cholesteryl ester mass transfer. Plasma NCET (21.1 ± 1.8 nmol/ml/hr, N = 13, mean ± SD) and plasma cholesteryl ester mass transfer (22.3 ± 1.9 nmol/ml/hr, N = 13, mean ± SD) were not significantly different in the subjects. In the two hemodialysis patients, plasma NCET rates were 24.3 nmol/ml/hr and 15.5 nmol/ml/hr and the corresponding plasma cholesteryl ester mass transfer rates were respectively 21.2 nmol/ml/hr and 16.5 nmol/ml/hr. Plasma LCAT activities measured in triplicate by the method of Channon and coworkers [22] in these two patients were 55.8 nmol/ml/hr and 51.1 nmol/ml/hr, respectively. Plasma NCET rate assayed in pentuplicate in a fresh normolipidemic plasma sample (26.1 ± 2.9 nmol/ml/hr, mean ± SD) and in three hypertriglyceridemic plasmas (73.5 ± 13.2 nmol/ml/hr, mean ± SD of 3 pentuplicate measurements) was not significantly different compared with rates in an aliquot of plasma which had been stored at −80°C for one week (normolipidemic plasma: 26.2 ± 1.8 nmol/ml/hr, mean ± SD; hypertriglyceridemic plasmas: 73.2 ± 14.8 nmol/ml/hr, mean ± SD of three pentuplicate measurements).

The data in Table 2 show that plasma NCET rates and apoB concentrations were significantly higher in CAPD patients compared with control values. ApoB levels in these patients were also significantly higher than values in renal patients receiving other treatments. Plasma HDL_3-C concentration was significantly higher in renal transplant recipients compared with levels in dialysis independent patients with chronic renal failure or those treated by CAPD.

Cholesterol esterification rates in whole blood were not significantly different in renal transplant recipients (56 ± 17 nmol/ml/hr, N = 17), patients treated by hemodialysis (42 ± 15 nmol/ml/hr, N = 12) or CAPD (44 ± 22 nmol/ml/hr, N = 11) and dialysis independent patients with chronic renal failure (41 ± 26 nmol/

Table 3. Plasma newly synthesized cholesteryl ester transfer (NCET) rate in normolipidemic and hyperlipidemic patients with renal disease and control subjects

	Plasma NCET rate *nmol/ml/hr*						
	Chronic renal failure					*P*	
	DI	HD	CAPD	RT	Controls	Treatment	Lipids
Normolipidemic (TC < 6.5 mmol/liter TG < 2 mmol/liter)	20.7 ± 2.7 *N* = 4	19.5 ± 4.2 *N* = 6	22.0 ± 3.6 *N* = 3	22.9 ± 6.2 *N* = 8	18.0 ± 5.6 *N* = 15	NS	0.003
Hyperlipidemic (TC ≥ 6.5 mmol/liter TG ≥ 2 mmol/liter)	27.6 ± 9.7 *N* = 9	29.2 ± 13.0 *N* = 6	36.3 ± 12.5 *N* = 8	32.4 ± 11.7 *N* = 9	27.3 ± 7.6 *N* = 12	NS	0.003

Values are mean ± SD. Significance is obtained from one-way analysis of variances (treatment) or two-factor analysis of variances (normolipidemic versus hyperlipidemic groups). Abbreviations are: NCET, newly synthesized cholesterol ester transfer; DI, dialysis independent; HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplant; TC, total cholesterol; TG, triglycerides; NS, not significant.

Table 4. Plasma newly synthesized cholesteryl ester transfer (NCET) rate, clinical characteristics and plasma lipid, lipoprotein and apolipoprotein concentrations in patients with renal disease with ischemic heart disease (IHD[+]) and those with no evidence of ischemic heart disease (IHD [−])

	IHD [+] *N* = 11	IHD [−] *N* = 42	*P*
NCET rate *nmol/ml/hr*	26.0 ± 5.4	27.8 ± 11.6	NS
Age *years*	65 ± 7	45 ± 16	<0.001
BMI kg/m^2	25.2 ± 3.0	24.4 ± 3.3	NS
Duration of treatment *months*	15 ± 14	53 ± 51	<0.001
Creatinine *μmol/liter*	580 ± 204	435 ± 374	NS
TC *mmol/liter*	6.98 ± 0.98	6.43 ± 1.66	NS
VLDL-C *mmol/liter*	0.62 ± 0.37	0.79 ± 0.61	NS
LDL-C *mmol/liter*	4.93 ± 0.83	4.24 ± 1.28	<0.05
HDL-C *mmol/liter*	1.33 ± 0.36	1.34 ± 0.43	NS
HDL_2-C *mmol/liter*	0.57 ± 0.35	0.56 ± 0.28	NS
HDL_3 *mmol/liter*	0.77 ± 0.14	0.79 ± 0.20	NS
TG *mmol/liter*	1.72 ± 0.62	1.93 ± 0.94	NS
VLDL-TG *mmol/liter*	0.94 ± 0.48	1.24 ± 0.79	NS
ApoA1 *g/liter*	1.27 ± 0.18	1.33 ± 0.26	NS
ApoB *g/liter*	1.17 ± 0.23	1.01 ± 0.36	NS

Values are mean ± SD. Abbreviations are: BMI: body mass index; TC, total cholesterol; VLDL-C, very low density lipoprotein cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; VLDL-TG, very low density lipoprotein triglycerides.

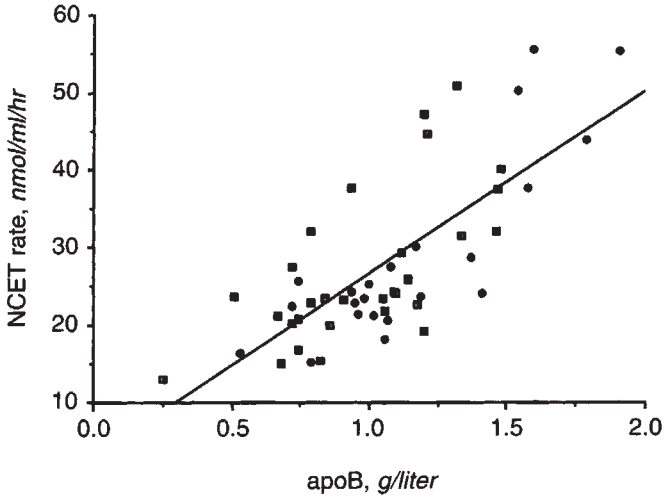

Fig. 1. *Correlation between plasma newly synthesised cholesteryl ester transfer (NCET) rate and plasma apoB concentration in patients with chronic renal failure or renal transplants.* Symbols are: (●) hemodialysis ($N = 12$, $r = 0.760$, $P < 0.01$); (○) CAPD ($N = 11$, $r = 0.915$, $P < 0.001$); (■) renal transplant recipients ($N = 17$, $r = 0.653$, $P < 0.01$); (□) dialysis independent patients with chronic renal failure ($N = 13$, $r = 0.662$, $P < 0.05$). $r = 0.754$; $P < 0.001$.

ml/hr, $N = 13$) compared with rates in a separate group of healthy subjects (55 ± 15 nmol/ml/hr, $N = 8$). These rates were significantly ($P < 0.05$) lower in hemodialysis patients compared with renal transplant recipients. Cholesteryl ester accumulation in incubated blood was determined as part of a separate study.

Table 3 shows plasma NCET rates in subjects divided arbitrarily into normolipidemic (cholesterol < 6.5 mmol/liter and triglycerides < 2.0 mmol/liter) and hyperlipidemic (cholesterol ≥ 6.5 mmol/liter or triglycerides ≥ 2.0 mmol/liter) subgroups. Plasma NCET rates were not significantly different by treatment in the normolipidemic subgroup or the hyperlipidemic subgroup. Rates of plasma NCET were significantly higher in hyperlipidemic subjects compared with normolipidemic subjects.

Renal transplant recipients treated with azathioprine and prednisone had plasma NCET rates (29.8 ± 12.3 nmol/ml/hr, $N = 5$, mean ± SD) which were not significantly different from rates in corresponding patients treated with azathioprine, prednisone and cyclosporin A (27.1 ± 10.1 nmol/ml/hr, $N = 12$, mean ± SD). Patients with both renal disease and ischemic heart disease were older with higher levels of LDL-C and similar rates of plasma NCET compared to those with no evidence of ischemic heart disease (Table 4).

Correlational analyses

Plasma NCET rate was correlated significantly ($r = 0.754$, $N = 53$, $P < 0.001$) with plasma apoB concentration in all renal patients combined (Fig. 1) and control subjects ($r = 0.477$, $N = 27$, $P < 0.05$). Also plasma NCET was correlated significantly with plasma cholesterol ($r = 0.692$, $N = 53$, $P < 0.001$) and triglyceride ($r = 0.664$, $N = 53$, $P < 0.001$) concentrations but not with age ($r = 0.087$) and BMI ($r = 0.116$). When plasma cholesterol concentration was held constant in partial correlation analysis, the correlation between plasma apoB concentration and NCET rate in renal patients remained significant ($r = 0.415$, $N = 53$, $P = 0.001$). Similarly plasma NCET rate was correlated significantly with plasma apoB concentration when plasma triglyceride levels were held constant ($r = 0.616$, $P < 0.001$).

Discussion

Our data show that hyperlipidemia is associated with raised plasma NCET rates in patients with renal disease and is essentially normal in normolipidemic patients irrespective of the treatment used to control uremia. Also plasma NCET rate was closely associated with plasma apoB concentration and high transfer rates of newly synthesised cholesteryl esters from HDL to apoB-containing lipoproteins may be linked with the hyperapobetalipoproteinemia of CAPD patients.

In vivo cholesteryl ester transfer is influenced by several factors including composition and levels of plasma lipoproteins, CETP concentration and transfer protein inhibitor levels. The plasma NCET assay used in the present study incorporates several of these factors and is a measure of cholesteryl ester flux between HDL and apoB-containing lipoproteins in the presence of native lipoproteins and LCAT activity. Moreover, our data show that plasma NCET rates are similar to rates of plasma cholesteryl ester mass transfer and values in healthy subjects were comparable with rates reported in previous studies [15, 22, 24, 27–31].

Plasma NCET rates depend on plasma LCAT activity and cholesteryl ester mass transfer. Thus low rates of plasma NCET would be expected in normolipidemic hemodialysis patients and undialyzed patients with chronic renal failure as judged by previous reports of low cholesteryl ester mass transfer rates and LCAT activity in the former [8] and low LCAT activity in the latter [32]. However, in the present study, plasma NCET rates in these categories of renal patients were similar to values in the healthy controls. Normal NCET rates could theoretically result from reduced plasma LCAT activity coupled with a counterbalancing increase in cholesteryl ester mass transfer. The tendency toward lower cholesteryl ester accumulation in incubated whole blood suggests that plasma LCAT rates in the undialyzed and hemodialyzed patients with renal failure might be lower than normal but not as low as the approximately one-third normal rates in hemodialysis patients reported by Dieplinger, Schoenfeld and Fielding [8]. On the other hand, plasma LCAT activity was not abnormally low and the rate of cholesteryl ester mass transfer did not appear to be abnormally high and was similar to NCET rates in two virtually normolipidemic hemodialysis patients we studied. Furthermore, normal plasma LCAT activity has been reported in patients treated by hemodialysis [32], indicating that low LCAT activity is not a consistent feature in these patients. It is possible that plasma LCAT activity was not sufficiently low to appreciably reduce plasma NCET rates in undialyzed or hemodialyzed patients with chronic renal failure.

The reason for the apparent discrepancy between the present findings of normal NCET rates and reported data indicating low cholesteryl ester transfer in hemodialysis patients [8] is not entirely clear. The patients in the previous study were considerably lighter [8] and were probably on a restricted diet. It is possible that low nutrient intake and specifically low cholesterol intake reduces plasma CETP concentration and cholesteryl ester transfer. Changes in dietary cholesterol are linked with changes in plasma CETP and CETP mRNA levels in humans [33]. Also, dietary intake influences plasma cholesterol levels which are associated with plasma CETP concentration [34]. Thus low plasma cholesterol levels (mean, 3.42 mmol/liter) in the hemodialysis patients studied by Dieplinger and coworkers [8] are in accord with low nutrient intake and reduced levels of plasma CETP and cholesteryl ester transfer. The low plasma LCAT activity in these patients [8] may also be due to low nutrient intake since a recent study has shown that a low-calorie diet reduces cholesterol esterification in plasma [35]. The markedly higher body weight in the current hemodialysis patients is undoubtedly due to higher nutrient intake which is consistent with higher levels of plasma cholesterol, CETP, cholesteryl ester transfer and finally NCET.

Plasma cholesteryl ester transfer and LCAT activity are reported as essentially normal in normolipidemic CAPD patients [8]. Our data are in line with these findings and showed normal rates of plasma NCET in the small number of CAPD patients with plasma lipids in the arbitrarily defined normal range. The elevated plasma NCET in the total group of CAPD patients was therefore due to the presence of hyperlipidemic individuals. An increase in plasma NCET with hyperlipidemia is consistent with raised plasma cholesteryl ester mass transfer documented previously in hyperlipidemic subjects [15, 16].

In renal transplant recipients accelerated plasma NCET rates were associated with hyperlipidemia which was probably due at least in part to the immunosuppressive therapy these patients were receiving. Increases in all lipoprotein fractions have been reported at some stage during prednisone treatment [36, 37] and cyclosporin A raises plasma LDL levels [12]. Corticosteroid therapy is also associated with a reduction in plasma CETP in both normal subjects and in patients with nephrotic syndrome [17] and with reduced liver CETP mRNA and plasma CETP in CETP transgenic mice [3]. However, in spite of the fact that all the renal transplant recipients in the present study were treated with prednisone, plasma NCET rates tended to be slightly higher in normolipidemic transplant patients compared with controls. We cannot rule out the possibility that azathioprine, a component of immunosuppressive therapy, raises plasma NCET rates which are then normalized by concurrent corticosteroid therapy. It seems unlikely that treatment with cyclosporin A influences plasma NCET rates which were comparable in patients treated with triple therapy or azathioprine and prednisone. Another possibility is that plasma CETP concentrations may not be rate-limiting for cholesteryl ester transfer in patients with kidney grafts. Overall it appears that plasma NCET rates are not abnormal during immunosuppressive therapy unless hyperlipidemia develops.

A major finding of the present study was the close association between plasma NCET rates and apoB concentrations in renal patients. The mechanism underlying this correlation is uncertain and may involve a direct effect of CETP on apoB levels and/or an effect of varying levels of apoB-containing lipoproteins on plasma NCET. A previous study has reported a correlation between plasma CETP concentrations and apoB levels in patients with nephrotic syndrome [17]. Thus it is conceivable that apoB levels are related to CETP concentrations and subsequently to NCET rates in the present renal patients. CETP might directly increase apoB levels by altering the formation and/or catabolism of LDL [38]. Furthermore evidence for a direct effect of CETP on plasma apoB levels has been recently obtained from studies using transgenic mice which showed an increase in plasma apoB levels when primate CETP was expressed [39]. Plasma levels of apoB-containing lipoproteins and particularly triglyceride-rich particles are acceptors of cholesteryl esters transferred from HDL, and are therefore an important determinant of plasma cholesteryl ester mass transfer [15] and NCET rates which were closely correlated

with plasma triglycerides in our data. However, the correlation between plasma NCET rate and apoB concentration was independent of plasma triglyceride levels, suggesting that the capacity of apoB-containing lipoproteins for accepting cholesteryl esters may not be the major factor underlying the correlation.

Hyperapobetalipoproteinemia has been previously identified in patients treated by CAPD [40], but the factors responsible for this abnormality are not clear. The elevated levels of plasma apoB in the present CAPD patients are consistent with the presence of hyperapobetalipoproteinemia. In addition plasma NCET rates were raised which coupled with the correlation between plasma NCET rate and apoB concentration suggest that accelerated cholesteryl ester transfer may be linked with hyperapobetalipoproteinemia in patients treated by CAPD.

Elevated plasma apoB levels have been associated with CHD in several studies [41–43], and raised plasma NCET rates have also been reported in patients with angiographic evidence of coronary artery disease [27]. However, in the present study, plasma apoB concentrations and NCET rates were not predictors of overt ischemic heart disease (IHD) in renal patients. Instead, established risk factors including older age and higher LDL-C levels distinguished the affected individuals. Nevertheless, plasma NCET rates in renal patients were similar to levels reported previously in patients with angiographic evidence of IHD (27.8 ± 12.2 nmol/ml/hr) [27], possibly reflecting the increased risk of IHD in patients with end-stage renal disease. These findings require testing in a larger sample of renal patients.

There are limitations to the present study. The study was cross sectional and does not take into account pretreatment levels of measured variables. Also the comparatively small number of renal patients in each category increases the risk of a non-representative sample. A heterogenous group of patients was deliberately chosen to reflect the current clinical pattern seen in renal failure rather than preselecting for different risk factors. The exception being the exclusion of diabetic nephropathy or the nephrotic syndrome with well-documented lipid abnormalities which would have further complicated the results. The patients with renal disease were taking a variety of medications. While most of the drugs prescribed were not known to affect lipid metabolism (apart from those taken by renal transplant patients), an effect of those agents on plasma NCET rate has not been positively excluded. Finally, dietary intake was not controlled and may influence plasma NCET rates and lipids, lipoproteins and apolipoprotein concentrations.

In conclusion, this study shows that plasma NCET rate is closely associated with plasma apoB and lipid levels in patients with renal disease and is elevated similarly with hyperlipidemia in both renal patients and subjects without renal disease. The data also suggest that raised plasma NCET rates may not be the inevitable consequence of renal disease and its treatment because transfer activity is normal when hyperlipidemia is absent. Whether or not reduction of plasma lipids by drug treatment and diet also reduces NCET rates in hyperlipidemic patients with renal disease warrants further study.

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